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BASOPHILIC AGGREGATIONS*

By ROBERT A. GLENN, M.D.

Samuel Merritt Hospital, Oakland, California

It is probably incontestably true that in no branch of the medical arts are such meticulous precision, such inflexibility of result so rigidly exacted as in the Clinical Laboratory. The Roentgenologists may wrangle over their visionary interpretations of mystical shadows on highly sensitive photographic film and the medical and surgical consultants may disagree over the evaluation of reluctantly publicized confessions dragged from an excoriated and victimized patient but the data submitted on an official clinical laboratory form, inappropriately, but all too frequently tinctorially designated a dubious blue, must be incontrovertably a symbol of mathematical exactitude.

It is no wonder, then, that we as laboratory workers, suffused by this beautitudinous atmosphere, acclaim with delight any method or procedure which promises to assist us to attain such worthiness. Perhaps even some of you can recall the comforting satisfaction that was ours when the God-given Kolmer first blossomed in our serological chaos. Prior to this there were as many ways of doing Wassermanns as there still are housewives' recipes for baked beans or lemon pie.

Read before the American Society of Medical Technologists, San Francisco, Calif., June, 1938.

Particularly on the Pacific Coast we feel much the same about Kofoid's staining method for parasites. We recall with chagrin the warm stools, hot footed to the laboratory in thermos containers which with almost parental devotion we carefully nourished on warm stages provided by ennumerable and ingenious gadgets.

And to the pathologists most certainly, has come such clarifying and enlightening assistance as is exemplified in Broder's Cancer Grading, enabling him to render an opinion of malignancy precise even to the exact numerical integer—of hopelessness.

It is, therefore, with inspired avidity that we accept the enumeration of Basophilic Aggregations as an index of the severity of lead intoxication. It was in 1924 that the results of the first use of this procedure were presented by McCord and his associates, but punctate or basophilic stippling of erythrocytes had been a subject of controversy since it was first noted by Ehrlich in 1885. The first correlation of stippling with lead poisoning was made by Behrend in 1899. A voluminous literature on the subject accumulated, which when summarized by Pappenheim in 1919, fell far short of proving the mechanism of its production, or of a clear-cut clinical conception of its significance. The toxic conditions in which stippling was found were well known; but regarding the granules themselves, opinions varied from the toxic granular degeneration theory of Pappenheim to the nuclear fragmentation theories of Koch and others. For our modern conception of this phenomenon we are indebted to the masterful report of Key who in 1924, working with rabbits in experimental lead poisoning, proved that the percentage of stippled cells rose and fell in direct proportion to the reticulocyte variation, and that the number of reticulocytes was approximately equal to the combined number of stippled and basophilic erythrocytes, thus proving that stippled cells were immature erythrocytes.

From this and other work later reported from many sources it is now accepted that in normal adult human life, the content of erythrocytes in the blood stream is maintained on a fairly uniform level by the orderly entry of new cells from the bone marrow replacing those that have been destroyed. These new cells are essentially mature, only about 1% exhibiting any of several known characteristics of immaturity. Under conditions in which toxic agents exert an action on bone marrow, such as lead, benzol, tuluol, etc., and

under other conditions in which physiological demands are made such as hemorrhage or the effects of high altitudes, increased numbers of red cells enter the blood stream. The chief characteristic of these liberated immature cells is the presence of basophilic substance on one form or another.

Polychromasia, punctate stippling and reticulation are but different manifestations of one phenomenon—the presence of basophilic substance. The exact form of this basophilic substance existing in the unaltered blood is little known. Probably the picture observed as polychromasia after staining is nearest to the natural state of this material. Disturbing though it may be, from all available evidence the impression is borne in upon us that reticulated cells probably are produced only as a result of laboratory manipulation and thus, while not being true artifacts, are actually laboratory creations. Through variations in laboratory technique there have been produced at will in a single blood smear all the well known varieties of reticulation. However, stippled cells are believed to exist as such in the unaltered blood. Through unknown processes the ultramicroscopic particles observed as polychromasia, or the basophilic materials in acid solution, are caused to arrange themselves into masses characteristic of stippling. These concepts are abundantly substantiated by numerous publications.

Basophilic substance has long been regarded as the foremost blood finding in lead poisoning, but to an overwhelming extent reliance has been placed upon the determination of the presence of stippled cells. The recognition, however, that polychromatophilia, punctate stippling and reticulation are but different aspects of the same material, now prompts us to place greater diagnostic dependence upon examinations for the totality of erythrocytes bearing basophilic material. Of the three forms of basophilic substance, stippled cells are the least common. The positive diagnostic significance widely attached to the qualitative presence of stippled cells in suspected lead poisoning is becoming more and more questionable; conversely less uncertainty is believed to attend the quanitative determination of all basophilic erythrocytes. The latter few paragraphs above have been rather freely paraphrased from recent literature.

The Test Method

The search for basophilic forms is best made in laked cells. During this process some of the basophilic material probably leaves the cell with the hemoglobin, but the remainder being insoluable in water, salines and some stains collect in masses, strands and reticula as laboratory artifacts. Such forms are far more visible than polychromatophilic cells and more numerous than stippled cells. It was to these laboratory creations that McCord first applied the term "Basophilic Aggregations."

Thin, even blood smears are made and allowed to dry: The proper drying of these smears becomes important. If allowed to become excessively dry, that is over 12 hours, some of the basophilic containing cells will not lend themselves to aggregation and on the other hand insufficient drying facilitates removal of whole cells during the staining process. Ordinarily the optimum time lies between one and three hours. Each locality must be appraised by trial and error with due consideration for present temperature and humidity. Here on the Pacific Coast and particularly in the Bay Region where no great shifts in temperature or humidity are ordinarily experienced the usual optimum drying time is about 2 hours. In this connection it is interesting to note that definite evidence has been offered that basophilic aggregation in normal individuals exhibits a physiologic daily rhythm which coincides with the daily meteorologic alterations, the observed peaks being in accord with points of high barometer and low temperature. Since these reports seem to emanate chiefly from the cyclone, hurricane and dust storm belt of the Middle West we are happy to note that the Barometer in the Bay Region registers a monotonous 763, almost the year around.

After drying, one half of the slide is overlaid lengthwise by a strip of filter paper previously dipped in methyl alcohol. This is allowed to dry until the filter paper curls itself loose from the slide. The whole slide is now immersed in a Coplin staining jar containing Manson's Stain for approximately 10 minutes. The formula for the Manson's Methylene Blue stain is as follows:

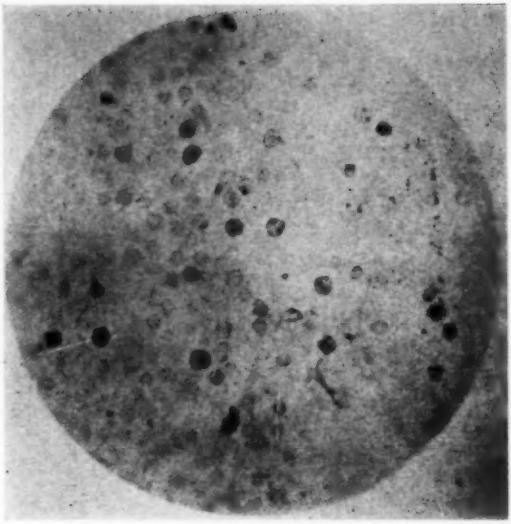

Fig. 2. Microscopic field from laked portion of slide presenting numerous basophilic aggregations, in blood of highly exposed lead worker.

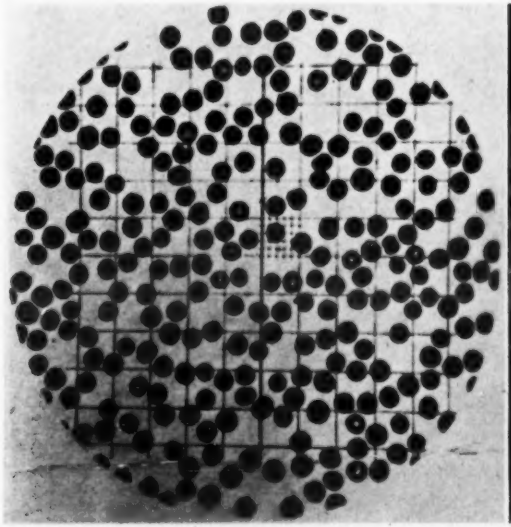

Fig. 3. Microscopic field from fixed portion of same slide as in Fig. 2 showing inlaked stained erythrocytes and Whipple grid for guide in counting.

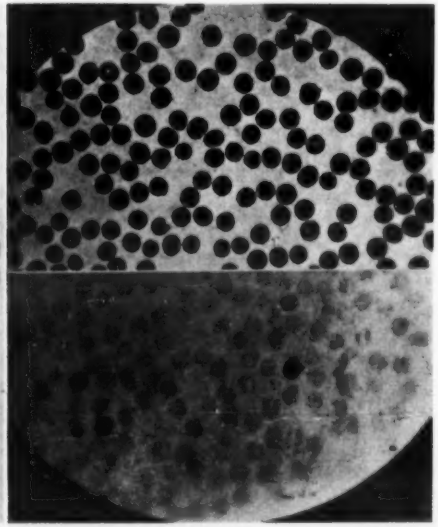

Fig. 4. Schematic contrasting of appearance of fixed and laked microscopic fields with few basophilic aggregations in laked portion.

MANSON'S METHYLENE BLUE STAIN

Borax.....0.4 to 5 gms.

Boiling Distilled Water.....100 c.c.

Methylene Blue .....2 gms.

The sodium borate is dissolved in the boiling distilled water and to this is added the methylene blue. After filtering this stain is ready for use and is stable and uniform for about 2 weeks. After longer use a precipitate may appear. In a more recent publication McCord now recommends that in the preparation of the Manson stain best results will be attained if the boiling water to which the borax is added is allowed to cool to room temperature before the addition of the methylene blue. Our own experience has been that the whole formula is best handled according to test results. Thus in our hands about 2.5 gms. of borax is required with the average methylene blue powder purchased in the open market.

It cannot be too forcefully emphasized that thin, even smears be prepared. Thick smears make enumeration difficult if not impossible. The time of staining is not arbitrary and a readable stain may be obtained in two minutes. After staining, it is necessary to wash the slides through three or four rinses of distilled water.

We now have a slide half of which is fixed and half laked, the whole stained a deep blue. The slide shows the schematic contrasting of fixed and laked microscopic fields with few basophilic aggregations in the laked portion. The usual microscopic arrangements include an oil immersion objective and a 10x ocular which is fitted with a Whipple grid. The outer lines of this grid determine the microscopic field. The average field in a good preparation contains approximately 150 red cells. Before counting, the slide should be examined for an area showing a suitable distribution of the red cells. Any comparable or equitable plan of counting may be adopted. Our own plan is to count four average fields in the fixed portion of the slide the distribution of red cells in which is about 150 cells each. Above this number the cells are too concentrated for accurate

the development of clinical lead poisoning is a fair expectancy. In our experience, particularly valuable, in this latter connection, has been the periodic re-examination of the same workers under the same industrial conditions with comparative recordings of their findings. We have been able by this means in repeated instances to detect the incipience of otherwise undetectable lead intoxication.

BASOPHILIC AGGREGATE EXAMINATIONS

	Jan. 1935	Dec. 1935	June 1936	Jan. 1937	July 1937	Jan. 1938
Whittier, F. A.....	0.2	0.66	0.21	0.16	0.34	0.22
Hollinger, M.....	0.35	0.33	0.10	0.55	0.46	0.29
Fickes, L.	0.5	0.65	0.6	0.5	0.77	0.48
Pidgeon, G.	0.15	0.80	0.4	0.2	0.47	0.41
Amoroso, A.....	0.15	0.39	0.2	0.33	0.15	0.4
Norton, C. F.....	0.25	0.34	0.5	0.45	0.34	0.19
Sherry, J. 0.2		0.6	0.19	0.22	0.13	0.61
Simmons, G. W.....	0.3	0.5	0.22	0.08		
Putnam, R.	0.12	0.5	0.12	0.2	0.45	0.24
Reese, A.	0.2	0.56	0.14	0.8	0.58	0.43
De Cuis, C.....		0.16	0.29	0.2	0.2	0.21
Patrick, E.		0.35	0.38		0.59	0.34
Davis, G.		0.29	0.28	0.22		
Hill, R. W.....		0.85	0.26	0.4	0.54	0.53
Kelly, J.			0.15	0.8	0.37	0.13
Truscott, R.				0.35	0.21	0.32
Moncher, R.					1.04	0.69

In all honesty it must be stated that as lead poisoning progresses to extended chronicity, the worth of this procedure diminishes. There appears to be an unmistakable recession of the number of basophilic aggregations even in the presence of frank manifestations of chronic plumbism. Under such conditions punctate stippling may still persist.

Here, then, is submitted a relatively simple yet precise method of expressing by a numerical integer what appears to be the red blood cell reaction to lead intoxication. In our experience it has

counting and are bound to overlap, etc. For each field of red cells counted in the fixed portion ten fields are counted in the laked portion. The basophilic aggregations thus counted are then expressed as a percentage of the red cells counted in the fixed portion. Thus if 76 Basophilic Aggregations were found in the same compared areas in which say 4000 red cells were counted the obvious percentage is 1.9.

It is appreciated that a little practice is required to assure familiarity with the method and hence insure accuracy.

We have said previously that the blood of normal human adults rarely contains more than 1% of basophilic erythrocytes, the average in our experience lying probably between 0.4% and 0.8%. With workers absorbing lead without clinical manifestations and in early lead poisoning the percentage commonly ranges from 1.5% to 4.0% with occasional findings much higher. The zone between 1.0% and 1.5% may be accepted as representing the threshold, findings within which being open to doubt but at least acceptable as warnings. In the absence of other pathology, any finding in known lead exposed workers of, or in excess of 1.5% at once suggests the probability of lead absorption. Findings in excess of 2.0% or 3.0% are to be associated with an increased imminence of clinical lead poisoning.

With these fairly well fixed standards in mind it is clear that several usages of the method are applicable. In lead exposed persons, otherwise in normal health, the detection of basophilic red cells in concentration in excess of 1.5% and particularly in excess of 2%, suggests lead absorption and the possibility of approaching clinical lead poisoning. Two weeks of lead exposure appears to be ample time to induce such diagnostic accretions.

The mass investigation of all the workers in the several departments of an industrial plant permits the rating of the divers departments as relative hazards to the workmen. Under these circumstances also the results may very well be used as a guide directing attention to those departments requiring correctional measures for the reduction of such industrial hazards. Finally by this type of investigation we have a means of identification of those workers for whom medical care is necessary and of those workers in whom

proved a most satisfactory addition to our diagnostic armamentarium.

Since the material for this presentation was accumulated an additional publication on this subject has appeared in "Industrial Medicine", April, 1938, by Hyler and Bradley of Detroit who studied, by this method, the incidence of lead intoxication in 390 workers in the body finishing department of an automobile plant where a lead exposure was proved to exist and where lead poisoning reached epidemic proportions.

This paper is concluded by the following verbatim quotations from this article:

"The need for early evidence of lead absorption and incipient lead poisoning in an attempt to obviate the disability accompanying frank lead poisoning has prompted many inquiries seeking simple procedures that readily may be applied to large numbers of workers engaged in lead using occupations.

"There are valid reasons to believe that a series of quantitative determinations of lead present in urine and feces may afford the most precise information in this situation. However, the laborious quantitative determination of lead in such excreta is far from practical when the number of workers repeatedly to be examined at short intervals is represented by several hundreds or thousands.

"From the standpoint of time, economy, accuracy, uniformity and simplicity, a preference is expressed for the McCord Basophilic Aggregation Test in the examination of workers when seeking the detection of lead absorption and early lead intoxication."

THE EFFECT OF TIME AND TEMPERATURE OF STORAGE ON THE RESULTS OBTAINED IN THE BACTERIOLOGICAL ANALYSIS OF WATER

By THEODORE W. KEIPER, M.A., M.T., now with Dr. Ferris, Haden & Lindberg, 115 S. Stone Ave., Tucson, Ariz., and JOHN S. FLINN, B.S.
Pamsetgaaf Laboratory, Prescott, Arizona

Introduction

It is the practice of some laboratories to test samples of water sent through the mail, and then judge the quality of the water from their results. This means that the samples have been stored at room temperature from several hours to several days, before being tested. Whether or not a water supply can be judged *fairly* by such procedure, is an important question.

Historical

The writers first became interested in this question several years ago, when they learned that their results of testing the municipal water supply of the City of Prescott, did not agree with *some* of the results of another laboratory. Upon inquiry, it was learned that both laboratories were using standard methods. Laboratory procedures, then, did not explain these differences. Further inquiry revealed that the other laboratory tested samples received through the mail. To the writers, this fact explains the differences, as Standard Methods of Water Analysis, eighth edition, page 207, states "The time allowed for storage, or transportation, of a bacterilogical sample, between the filling of the sample bottle and the beginning of the analysis, should not be more than six hours for impure waters, and not more than twelve hours for relatively pure waters. During the period of storage, the temperature shall be kept between 6°C. and 10°C. Any deviation from the above limits, shall be so stated in making reports."

Purpose

It is not the purpose of this paper to criticize any laboratory's

after storage at room temperature for 24 and 48 hours. The average plate count in samples tested within one hour after collection, was 11. This elevated to 183 in 24 hours, and to 1,227 in 48 hours. If we were to judge the water supply according to the standards of the American Public Health Association, and U. S. Treasury, it would be as follows:

1. Testing within 1 hour after collection—safe for human consumption.

2. Testing after storage for 24 hours, at room temperature—unsafe for human consumption.

3. Testing 48 hours after collection, stored at room temperature—unsafe for human consumption—bad water.

In other words, a very good water becomes unsafe in 24 hours, and bad in 48 hours, when stored at room temperature.

The number of E. Coli, (confirmed), increased from 1 only, in all of the 100 samples when tested within one hour after collection, to 5 only, in 24 hours, and to 8 in 48 hours. Even with this increase, according to the preceding mentioned standards, the water would still be considered safe for human consumption. If, however, this same percentage of increase were maintained in samples with more positives to begin with, then if 10 confirmed E. Coli were present in the samples tested within one hour, this number would increase to 50 in 24 hours, and to 80 in 48 hours. By the preceding mentioned standards, the water would be considered unsafe.

In this series of tests, 50 samples, or 50%, showed an increase in the plate counts. Thirty-five samples remained practically the same, and 15% showed a slight decrease. The decreases occurred in samples with a plate count of less than 10; so they could really be considered as remaining the same. This shows that one cannot depend on the results of tests on water, when stored at room temperature between the time of collecting and the beginning of analysis. The increases in the plate counts were all definite, and in many cases quite marked. A typical sample was one collected on 1/31/39. The original plate count was 8. This increased to 500 in 24 hours, and to "too numerous to count" in 48 hours.

method of sampling water supplies, but to present some results on experimental work done on the effect of storage at room temperature, from the time of filling sample bottle to the beginning of the analysis.

Experimental

In order to subject the samples to the same conditions, (temperature), as the samples sent through the mail, in this study from May 30, 1938, to February 13, 1939, one hundred samples of water were tested by standard methods in the following manner:

1. Tested within one hour after collection (control).
2. Tested 24 hours after collection, stored at room temperature.
3. Tested 48 hours after collection, stored at room temperature.

Results

The results obtained are as follows:

Plate Counts:

1. Tested within one hour after collection—1,122, or an average of 11.2 per sample.
2. Tested 24 hours after collection—18,324, or an average of 183.2 per sample.
3. Tested 48 hours after collection—122,726, or an average of 1,227.3 per sample.

Number of Tube with Confirmed E. Coli:

1. Tested within one hour after collection..... 1
2. Tested 24 hours after collection..... 5
3. Tested 48 hours after collection..... 8

Types of Changes:

There was a small decrease in the plate counts in 15 samples; i.e., from 8 to 3 to 1, or 8 to 5 to 3, etc. In 35 samples the plate counts remained the same; i.e., 4-4-4. In 50 samples the plate counts increased. In some the increase was slight, and in some the increase was marked; i.e., 4 to 500 to "too numerous to count."

Discussion

The results obtained show a definite increase in the plate counts,

Conclusions

1. This shows conclusively, that the plate counts increase in 24 and 48 hours, in water samples stored at room temperature, between the time of collecting and the beginning of analysis.

2. Water supplies, stored for any length of time at room temperature, show an increase in confirmed E. Coli.

3. Although the number of samples tested is too small to draw any definite conclusions, they indicate that water samples stored at room temperature for any length of time before the beginning of the analysis, is NOT a reliable procedure for judging the character of a water supply.

THE MUSEUM IN A SMALL HOSPITAL*

By PHYLLIS STANLEY, M.A., M.T.

Presbyterian Hospital in Newark, N. J.

The aim of this discussion is to arouse interest in the preparation of exhibits and to demonstrate various methods of displaying pathological material suitable for a small hospital.

A museum may serve several purposes. First, it may be a depository where vast quantities of material and data are stored ready for reference. Second, it may be used to educate the Medical, Nursing, or technical staffs. Third, it may be a show place to interest and educate the laity. In a small hospital probably the two most common purposes are to save valuable scientific material and have it ready to present at a scientific conference or exhibit. I shall try and demonstrate the methods which we use in mounting gross specimens, preparing photographs and lantern slides in order to present a complete composite picture for such occasions.

A scientific exhibit requires scientific technic and as scientific people we do not appreciate unattractiveness. But the essential problem is the same in art, science, and medicine. An exhibit should be made intelligible and educational as well as attractive. The organization of material is important. The arrangement of the material in such a way that its value can be interpreted is essential. An exhibit need not show all that is known about a subject. It is important to select and choose wisely the most important facts and material. Often an exhibit is striking not by what it gives, but by what it leaves out. Material should be presented in such a way that the visitor can interpret it and realize its importance.

In preparing a large museum the arrangement of the room is important. A number of things should not be in the range of vision as they cause museum fatigue and visual conflict. The background should be of some plain neutral color. The lighting is very important as the visitor should be able to see clearly and easily.

Read before the American Society of Medical Technologists, San Francisco, Calif., June, 1938.

One of the most important things to accomplish in preserving gross pathological specimens is to keep the organs so that they retain their natural size, shape, and color. The technologist should begin to prepare the specimen as soon as possible after it is removed from the body. Operative specimens should be sent to the laboratory as soon as removed and their preservation started immediately. Whenever possible the technologist should go to the morgue and see the organs in the body and then prepare them so that they will be as near life-like as possible. Often it is hard to induce the pathologist or visiting surgeon that a tumor, lung, or brain should not be sectioned at the autopsy, but in the long run a much better permanent preparation is obtained. We prepare our specimens by the Kaiserling Method. This consists of three solutions. The first contains

Formalin	200 cc.	Potassium acetate	30 gms.
Water	1000 cc.	Potassium nitrate	15 gms.

A specimen is immersed in this solution until it is thoroughly fixed. The specimen is tied down, filled with fluid or cotton, and prepared so that the size and shape will be distorted as little as possible. The fixing process may take anywhere from a few hours to a few days depending on the size and consistency of the specimen. Then it is washed in running water for some time, untied or cut as desired, and placed in 95% alcohol until the color returns. It is then washed in running water and placed in Kaiserling III solution which contains

Potassium acetate	100 gms.
Glycerin	200 gms.
Water	1000 cc.

We keep our specimens in 5 gallon crocks until we are ready to mount them. Small specimens may be wrapped in gauze. A cardboard label is put on when the specimen is first put in K. I and replaced by a permanent label when it goes into K. III. Permanent labels may be made from thin white Du Pont Paraloid, inscribed with waterproof India ink.

Gross specimens may be stained with special dyes or solutions to bring out certain things. In my exhibit you will see a breast stained with Sudan IV (Scharlach R) so that the fat is stained and the cancer tissue remains white. After the specimens have been in

K. III a few days, they are ready to be mounted. Each specimen should be trimmed of excess tissue and fat and a smooth clean-cut surface should be next to the front if a cross section is to be shown.

The specimen is mounted on a black, white, or transparent background, depending on which shows up the important features best. I use linen thread or black silk such as is used in the operating room, and slightly curved sharp needles to sew the specimens to the background. Small tabs made from old films are sewed on the back to tie the strings through, so that the specimen will not slip. After the specimen is placed in a musuem jar of proper size, it is washed with running water to remove any small, loose particles and then allowed to stand for a time in K. III. This is changed and any air bubbles are brushed off the specimen. The top of the jar and cover are cleaned with soapy water, alcohol and Xylol, and melted museum jar cement is applied to the top of the jar. The cover is pressed on, heated lightly with a bunsen flame to spread the cement, and a weight is placed on top. I bind the top edge with waterproof adhesive and give it two or three coats of high gloss black paint. Uniform labels are an important item in making for a good-looking museum. We have one size printed on rather stiff paper and cut them down when the jar is small. We stick the labels to the jar with Sphinx paste made by the Arabol Co.

Photography is another phase in the preservation and presentation of material.

We use one stock size (5 x 7) for all negatives. This aids not only in standardizing the time and light for exposure, but also aids in developing and printing. Of course lantern slides are 3¼ x 4 and a set of equipment for exposing, developing, and preparing them is necessary.

I shall not go into detail about photographic methods. Various methods are shown in detail in the exhibit. I use a Clinical camera with a 5 x 7 back for taking pictures and a lantern slide back for reducing these negatives to lantern slides. I use a large photo micrographic apparatus with 5 x 7 plates for taking negatives.

For three color negatives I use 5 x 7 films and paper. For the gross photographs and clinical pictures we use two Sole lights each

with a 500 watt bulb and a larger lamp with a 1000 watt bulb. For the photo micrographs we use an electric bulb with a flat ribbon filament.

For Kodachrome transparencies we use a Leica or Contax camera on a home made stand over the microscope. There are all sorts of various little gadgets on the market which add to the expense and make the technic of taking a picture easier or more intricate as the case may be.

X-Rays may be reduced to 5 x 7 and printed on translight film (as demonstrated in the exhibit) or on paper or reduced for lantern slides.

I use a standard size in the transparency boxes and have a dye to cut the frames from black paper. After the initial cost of making the dye, the cost of having the frames cut is small. For mounting the various types of pictures I use one standard size glass and cardboard (6 x 8 inches). If transparencies are to be mounted separately, two pieces of glass or Kodaloid may be used. There are various styles of gummed paper to use for binding. Regular lantern slide binder is the cheapest. Half inch cellophane in red or black is effective. The rough crinkled Scotch tape can also be used, but it tends to get soft and sticky in warm weather and peel off.

Lantern slides are another important phase in the presentation of data for lectures, staff conferences, and exhibits.

The scope of the lantern slide is unlimited. Any negative of a clinical picture, gross specimen, or photomicrograph may be copied, enlarged, or reduced. Charts may be copied from case histories or books. Illustrations from books and equipment may be copied.

Cellophane may be used to make small charts, show briefs of histories or headings or aid in explanations. Colored cellophane may be used to show change of subject. Lantern slides may be colored by special crayons or transparent water color. Colored lantern slides may also be prepared from the color separation negatives, Dufay films or Kodachrome. Finlay preparations and Lumiere plates may also be used. Stained sections of specimens may be used to show low power magnification. The large specimens mounted on lantern slide plates are very effective.

The 35m. projector is one of the newer additions to the field of lantern slides. Colored pictures of patients, gross specimens, and photomicrographs are easily mounted either bound between glasses or in the metal frames. Kodachrome is easy to prepare as it only has to be exposed and then sent to the Eastman Co. who return the positive transparency. The field of the motion picture is boundless, both in black and white and color, and now sound pictures are being made of scientific subjects. But these require their own standardized cameras, processing tanks, solutions, and projectors. They are not usually included in the equipment of a small hospital laboratory.

METHODS OF COUNTING BACTERIA AND AN EXTENSIVE BIBLIOGRAPHY ON THE SUBJECT OF BACTERIAL COUNTING

By SISTER MARY GERARDA JONIK

College of St. Francis, Joliet, Illinois

Various methods for determining the viable or total count of bacteria have been advanced from time to time. These methods are important to research and practical bacteriology in any problem based on statistical analysis of the bacterial population. Such an analysis is necessary in the dairy industries to gauge the fitness of a milk supply, in health work to control the bacterial content of drinking water and to score sanitation of swimming water. It also has practical application in the industries which depend on bacterial by-products. In bacteriological research many basic problems concerned with an understanding of the manner in which bacterial cells live, multiply and survive depend on a knowledge of the number of cells present.

Since bacterial counting has such practical and theoretical use and since the literature on the subject is so scattered and diversified, an effort has been made to group the various methods and to present an extensive bibliography of extant works in this particular field. It is hoped that this grouping will aid research workers who are interested in devising new methods—methods more simple and accurate and which may be prepared with a minimum of expense and time.

Total Count Methods.—In these methods an attempt is made to estimate the total number of bacterial cells, living and dead, at a certain period of time. Macroscopic and microscopic means are employed for the purpose of counting total bacteria. If the technique is macroscopic the opacity of a mass of cells may be measured against a known standard (nephelometer, tyndallmeter, densitometer); the total cell volume may be measured by precipitating them (centrifuge or use of chemicals); the mass may be estimated by the amount of dye absorbed (methylene blue, etc.); the mass may also

be determined by the amounts of products produced (fermentation, etc.); or finally the growth of cells may be measured by the area covered by the growth. If it is microscopic the number of cells can be estimated by using ruled glass slides (counting chambers of various types) or against a previously determined standard (R.B.C.). Klein (1900) appears to have been one of the first to realize the value of a total count.

With the nephelometer, the opacity, i.e., the density of a bacterial suspension, may be measured in terms of light transmitted to the eye, by the tyndallmeter in terms of the intensity of the turbidity of the flux, by the densitometer in terms of the transmitted flux itself. The density may be measured by various photometric devices (photoelectric cells or a photographic plate), or by comparison with a set of previously determined standards (BaSO, or shaded black lines), or by comparison with the visibility of a platinum loop dipped below its surface.

The opacity method or some modification of it has been used by Wright (1900), Hopkins (1913), Brown and Kirwin (1915), Dreyer and Gardner (1916), Brown and Fisher (1919), Lambert (1919), Dunham (1920), Gates (1920), Holker (1921), Drennan (1923), Liese (1926), Schmidt (1926), Roubal (1927), Krueger (1929), Richards and Jahn (1933), Awtonomowa and Stessel (1934), Jennison (1934), Mestre (1935), and Longworth (1936).

Determination of the number of bacterial cells in a suspension may be made by measuring the amount of dye absorbed, the strength of a dye is determined by its color. Certain bacteria have the power to absorb the dye in greater or lesser amounts depending upon the number of bacteria present. This method was used by Hasting (1919), by Ellenberger, Band and Robertson (1928), and a modification of it by Thornton (1937).

In an attempt to furnish a method at once rapid, easy to use, and within the requirements for the standardization of vaccines, an effort has been made to measure the volume or the weight of the total number of cells in a certain volume of suspension by throwing them out of solution in the centrifuge. For this purpose capillary tubes or special centrifuge tubes are used and the volume of cells

read directly from the tube or the tube is weighed. This technique has been used by Baldwin (1927), Schmidt and Fisher (1930) and others.

A discussion of the different methods for counting bacteria, in so far as macro total counts are concerned, would be somewhat incomplete without reference to the estimation of the growth of a colony by the area it covers or the growth of bacteria in broth by increasing turbidity. While this is a method common to bacteriology no recorded attempt has thus far been made to make such estimation more accurate by instrumentation. No record is made here of investigators who have used such methods in their study of growth; they are too numerous.

The direct or micro total count method depends on counting the bacteria against a known area, such as the area of the objective field or a space marked off on a glass slide, or counting them against a known standard, i.e., red blood corpuscles, or by the hanging drop method. The counting chamber is most widely used for this purpose.

Direct counting methods in one form or another have been used by Wright (1902), Harrison (1905), Winslow and Willcomb (1905), Oram (1906), Prescott and Breed (1911), Harvey and Acton (1914), Breed and Brew (1916), Nelson (1917), Vincent (1918), Dreyer (1921), Supplee and Asbaugh (1922), Wilson (1922), Briedigan and Chang (1924), Smith (1925), Breed and Fellow (1929), Gray and Thornton (1929), Steiner (1929), Bernhardt (1932), Crowley and Richert (1935), Gehm and Heukelekian (1935), Ziegler and Halvorson (1935).

Some authors, Wright (1902), and Dreyer (1921) have substituted R.B.C.'s for the counting chamber claiming accuracy comparable to the results obtained with the counting chamber and a further advantage of greater ease of operation. Criticism has been directed by Smith (1925) and others against the use of R.B.C.'s on the basis of failure properly to standardize blood, on the loss of R.B.C.'s through rupture, or a failure to distribute bacteria and R.B.C.'s evenly on a slide.

Viable Count Methods.—The indirect or viable count may be performed by the following methods: (1) dilution, (2) dilution and

plating in agar, (3) estimating the amount of by-products formed, (4) by differential staining and (5) by the use of total count methods. The estimation of bacteria by the agar plate system of dilution is the usual method employed in attempting to count viable bacteria. This method is based on the assumption of Koch that every living cell is capable of producing a colony if placed in a suitable medium; therefore, every colony represents the number of viable organisms plated. The agar plate technique has been modified by Esmarch (1886) using "roll tubes", Frost (1916, 1920) his "little plates", Varney (1927) an "inoculating machine" and Reyniers (1935) "mechanized plating".

Esmarch (1886) attempted to simplify the agar plate technique by using the so-called "roll tube" method. By this process the culture was diluted in a tube of melted agar and rolled. As the tube was rotated the agar formed a thin film around the walls of the tube and hardened. This method was used by Wilson (1922) who claimed greater accuracy for it than was possible by the agar plate method. Corroboration of Wilson's results has been offered by Gullberg (1932). The technique has been modified by Kozlowski (1934) using a gelatin film culture; it has been simplified by Hunt (1932) so that the tube is spun in a machine, and by Redvers Thompson (1934) who designed a mechanical device for preparing and inoculating roll tubes.

With the idea of simplicity in method and equipment and of shortening the time element Frost (1916, 1920) has adapted the "little plate" technique for the estimation of viable bacteria. He diluted the bacterial cells, mixed them with small quantities of agar, spread them on a slide and allowed them to develop into colonies. These colonies were microscopic in size. Simmons (1919) found the "little plate" technique to compare favorably with the cultural plate method.

Present day methods frequently make it impossible to obtain accurate counts of bacteria on account of the difficulty in separating the colonies into discrete units. Varney (1927) has modified the agar plate technique by using the surface culture method in order to effect an isolation of the bacteria. By means of his inoculating machine the organisms are deposited in spiral streak formation upon

the agar surface. A special inoculating spud is used for this purpose, the streak is started on the outer portion of the medium and slowly drawn inward thus giving it a spiral form.

It is only in recent years that Reyniers (1935) has modified the agar plate method by the introduction of "mechanized counting". Briefly, his procedure consists of spreading ,by means of a special fine spreader, a diluted sample on the surface of an agar film, the colonies when developed show on one plane in regular formation somewhat similar to Varney's inoculation. However, Reyniers has introduced instrumentation wherever possible, i.e., he has mechanized all steps in obtaining a viable count, thereby eliminating the subjective factor which is the cause of discrepancies in most other methods.

Besides the foregoing essential modifications much has been done to simplify apparatus and steps in the plate method. In the step involving dilution automatic pipettes are used by Reyniers (1935) and Trexler (1935), dropping pipettes are suggested by Chick (1904), Donald (1905), Penfold (1919), Wilson (1922), and Smart (1926). Gotslich and Weigang (1895) suggest the use of special gravimetric methods; Fleming (1924), Smith (1924), Reyniers (1935), etc., describe the use of special devices for controlling the column of fluid in a volumetric pipette. Miller (1924) considers the accuracy of the platinum loop.

Since the entire success of the plate method depends on the accuracy with which the colonies can be counted many special aids have been suggested for this purpose. A ruled glass plate is used by Brunner and Zowadski (1893), Jeffers (1898), Thiele (1902), Brudny (1910), Schneider (1912), Buck and Swenarton (1926), Parker (1926); a projectoscope by Broadhurst and Brown (1925), Pesch (1931); a projectoscope and an "electric eye" by Reyniers (1935); special pens by Brudny (1910), Robinson (1930); contact printing on photographic paper by Lewis (1932); a hand tally by Wilson (1922); Quebec colony counter by Archambault (1937); a lumi-lens type illuminator by Butterworth (1936), an atomizer by Crowley and Rickert (1935), and an automatic colony counter by Varney (1935).

For many years bacteriologists have been using dilution methods to isolate species and to give some idea of the number of organisms present in the material examined. In this method a series of dilutions are made in broth and the highest dilution at which growth shows indicates the number of organisms in the specimen. Dilution methods have been used by Wilson (1907), Hill (1908), Muntsch (1929), and others to obtain direct counts. Wilson (1922) employed them for proportional counts, Tulloch (1905), Dreyer and Gardner (1916), Holker (1921) used series of dilutions to standardize vaccines. Ziegler and Halvorson (1935) made an experimental comparison of the dilution method, plate count and direct count.

Attempts have been made to estimate the growth of bacteria and hence the viable cells in a culture by measuring the amount of acid or gas produced, i.e., the rate of fermentation of a sugar by Rahn (1900), Allen (1917), Cullen and Chesney (1918), Northrup (1918), Martin (1919), Anderson (1924) and Langwill (1924). Since the extent of fermentation is assumed to be parallel to the bacterial growth, the fermentation capacity of a single average cell is first calculated and from this coefficient the number of cells present in a sample is estimated. This method can be used not only with organisms which ferment sugars but also with those that produce any by-product which can be measured.

Estimation of the bacterial population by differential staining methods may be used to count total or viable bacteria. The same is true of the total count method since any total count can be used to estimate either the viable count or the non-viable count. Differential staining methods have been employed by Fraser (1920), Koser and Mills (1925), Beattie (1927) and Bickert (1930).

This article has treated briefly the various methods of counting bacteria. The methods presented are by no means equal in importance or in practical value. While there is something of advantage in all of them not one of them can lay claim to perfection. It has not been the purpose of the writer to evaluate these methods or to pass criticism on any of them but simply to supply a survey of the experimental methods which have been used by research workers thus far. A more detailed account of these methods can be obtained from articles, the bibliography of which follows.

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METHODS FOR SECURING CLEAR SERA

By HENRY G. HADLEY, M.D.

1252 Sixth St., S. W., Washington, D. C.

In complement fixation and flocculation tests, a clear serum is more satisfactory as the tests are easier to read and there will be less doubtful reactions.

According to Boyd*, a sample of blood withdrawn 4 to 6 hours after the ingestion of a large amount of fat causes a distinct milkiness of the serum. Beraut** found that opalescence was due to an increased percentage of fat cholesterol and albumen. This opalescence increased after standing and he concluded that there was a colloid biological complex responsible for the change.

If the blood is withdrawn in the morning before eating, the blood serum will have a low percentage of fat cholesterol and albumen from the absorption of food and thus the appearance of opalescence will be somewhat prevented.

The next important precaution is to centrifuge as soon as the clot is formed and pour off the serum into another tube to preserve for testing, for if the serum remains in contact with the clot, hemolysis will take place to some extent and opalescent changes occur much more readily.

Conclusions

To secure clear serum free from opalescence and hemolysis, the blood should be withdrawn after a fast of 12 hours and the serum should be withdrawn from the clot as soon as possible.

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* Boyd, E. M. Jour. Biol. Chem., 1933, 101, p. 323.

** Beraut, P. J. E. Paris, Theses, 1929.

ABSTRACTS

FURTHER STUDIES OF SULFANILAMIDE: J. Y. Fores. Jr. P. I. Med. Assoc., vol. XVIII, No. 12, Dec., '38, p. 765.

Results showed that this drug was absorbed in four hours when given orally and in 15 min. parenterally. Two to three days were required to establish equilibrium. Excretion was found to be dependent upon kidney function. The author reports the results of sulfanilamide administration in a variety of types of infection with the conclusion that it is most valuable in streptococcus hemolyticus and in cases of gas-gangrene where the streptococcus is the secondary invader. In many other infections it was without value. Hematological findings due to toxicity were anisocytosis and achromia with a decrease in RBC and destruction of reticulocytes. Hemoglobin values were 60-70%. Sulphemoglobinemia and methemoglobinemia may develop from an overdose.

AN EVALUATION OF THE ROLE OF THE FOOD HANDLER IN THE TRANSMISSION OF AMEBIASIS: J. J. Sapero and C. M. Johnson. Am. Jr. Trop. Md., vol. 19, No. 3, May, '39, p. 255.

Observations made on navy men on ships and on shore leave where they were known to be served by infected food handlers is offered in support of the statement that food handlers are of little importance in the transmission of amebiasis.

GASTROSCOPIC OBSERVATIONS IN PERNICIOUS ANEMIA: R. Schindler and A. M. Serby. Arch. Int. Med., vol. 63, No. 2, Feb., '39, p. 334.

Gastroscopic findings and laboratory findings are given for 23 cases of pernicious anemia. The authors correlate these by assuming that in pernicious anemia there are really two distinct conditions present, one, the dysfunction of the cells which produce the "anti-anemic" factor and the other, which follows the first, a degeneration of the epithelium which does not necessarily heal when the deficiency is relieved.

PATHOLOGY OF THE TONSIL WITH STATISTICAL REPORT AND MICROSCOPICAL STUDY: A. C. Starry. Ann. Otology, Rhinology and Laryngology, vol. 48, No. 2, June, '39, p. 346.

A report of 8,516 pair of tonsils showed 7 cases of tonsillar tuberculosis, 5 of syphilis of the tonsils, two of malignant tumor, two benign tumor and one trichina. Other types of chronic changes are discussed. The author affirms these findings justify routine examination of all tonsils.

THE VALUE OF THE WELTMANN SERUM COAGULATION REACTION AS A LABORATORY DIAGNOSTIC AID; COMPARISON WITH THE SEDIMENTATION RATE: S. A. Levinson and R. I. Klein. Ann. Int. Med., vol. 12, No. 12, p. 1948, June, '39.

The authors cite evidence to show that this non-specific test may be used to distinguish between exudative processes and fibrotic processes or parenchymatous liver damage. It is influenced by fewer irrelevant factors than is the sedimentation rate. The technique is given.

PRIMARY POLYCYTHEMIA WITH LEUKEMIC MANIFESTATIONS: L. Kellman. Am. Jr. Dis. Child., vol. 58, No. 1, July, '39, p. 146.

A case report of a girl 6 years at the onset. She was followed 5 years with highest RBC 9,000,000 and highest WBC 77,500 with 82% lymphocytes. Complete remission for 3 years prior to writing is reported.

TOTAL, DIFFERENTIAL AND ABSOLUTE LEUKOCYTE COUNTS AND SEDIMENTATION RATES OF HEALTHY CHILDREN 4 TO 7 YEARS OF AGE: E. E. Osgood, R. L. Baker, I. E. Brownlee, M. W. Osgood, D. M. Ellis and W. Cohen. Am. Jr. Dis. Child., vol. 58, No. 1, July, '39, p. 61.

This article is part of a series of observations on the normal blood findings from birth through adult life. No significant age or sex differences were reported within this group.

THE BEHAVIOR OF ABNORMAL HUMAN THYROID TISSUE CULTIVATED IN THE LINDBERGH APPARATUS: N. C. Foot, L. E. Baker and A. Carrel. Jr. Exp. Med., vol. 70, No. 1, July, '39, p. 39.

Thyroid tissue removed surgically under aseptic conditions was kept alive as long as two months in the apparatus by means of a solution simulating blood injected into the artery. The effects of iodin, adrenalin, hormones, etc., were studied by adding them to the perfusate and taking biopsies at intervals.

THE CHEMISTRY OF VITAMIN E, IV. THE SYNTHESIS OF TOCOPHEROLS: L. I. Smith, H. E. Ungnade. J. Am. Chem. Soc., vol. 4, No. 3, July, '39, p. 298.

The possible structure of the substances involved in this synthesis is discussed and the synthesis described. The product was found to be biologically active in 20 and 100 mg. doses.

OTOGENIC MENINGITIS: T. Cawthorne, J. Lar. and Otol., vol. 56, No. 8, Aug., '39, p. 444.

Discussion of the pathology, bacteriology and method of spread of meningitis originating in ear infections with suggestions for prophylaxis and treatment. Drugs of the prontosil type have brought about a hopeful decrease in mortality.

A NEW TECHNIQUE FOR THE BLOOD SEDIMENTATION TEST: C. Brooks, J. M. A. S. A., vol. 9, No. 3, Sept., '39, p. 72.

This method uses a graduated tube with a stopcock at one end and the other end ground to fit hypo needles. It is rinsed with anti-coagulant and drained, then filled exactly to the mark directly from the vein, closed by means of the stopcock, set up vertically and read at intervals.

A NEW METHOD OF VERIFICATION OF LATENT HYPERVITAMINOSIS: M. Oshima, I. Ikegaki. Jap. Jr. Gastroenterology, vol. 10, No. 2, 3, 4, Dec., '38, p. 85.

Water soluble vitamins are not likely to accumulate sufficiently to be important but fat soluble may occur in excess. Since they are closely associated with the liver and occur in varying amounts in bile, the same criteria applied to liver function may be used as a gauge for hypervitaminosis.

BOOK REVIEWS

A TEXTBOOK OF BACTERIOLOGY: The Application of Bacteriology and Immunology to the Etiology, Diagnosis, Specific Therapy and Prevention of Infectious Diseases for Students and Practitioners of Medicine and Public Health by Hans Zinsser, M.D., Consulting Bacteriologist to the Peter Bent Brigham Hospital and the Children's Hospital, Boston and Stanhope Bayne-Jones, M.D., Professor of Bacteriology, and Dean, Yale University Medical School, Master of Trumbull College, Yale University, New Haven, Connecticut. Eighth Edition, Revised and Reset. Pp. 990. D. Appleton-Century Company, Inc., New York and London. Price ——

Many changes have been made in the eighth edition of this work which has long been a complete textbook of bacteriology. Large sections of the book have been entirely rewritten and others revised. The several sections are divided as follows: the general biology of bacteria and the general methods of bacteriology; infection and immunity; pathogenic micro-organisms; the rickettsia diseases; the spirochetes; medical mycology, the molds, yeasts, actinomycetes and pathogenic fungi; diseases caused by ultramicroscopic viruses, the exanthemata and diseases of uncertain etiology; technical methods of bacteriology, immunology and serology. In a review of this work it would be impractical to name or discuss the topics of the seventy-three chapters. Some of the new features of the volume may be mentioned however.

The main facts of bacterial variability have been generally accepted with recognition of many variants. Methods of studying variability and factors influencing variation are given. Substances required for growth and substances produced by growth have been newly determined by studies of bacterial metabolism. New facts have been added to our knowledge of the enzyme-systems of bacterial respiration and the relation of oxidation-reduction potentials to growth and activity. The toxins of diphtheria and tetanus have been highly purified and methods of production, concentration and standardization of their antitoxins are given. Improvements in pneumococcus typing and in serum therapy of pneumonia are dis-

cussed in detail. The serological grouping of streptococci has clarified many uncertainties in this phase of bacteriology. The chemical characterization of certain antigens has been accomplished and new methods for the selection and preservation of strains of organisms bearing the so-called "Vi" antigen have been found. Improved methods of cultivation of the Rickettsiae have been determined through intensive investigation resulting in the preparation of antigens and antiserums and a correlation of knowledge of relationships among the types of typhus and spotted fevers. Chemical studies of the viruses have yielded the indication that certain of them may be crystallizable proteins with autocatalytic properties. It has been determined that influenza is a virus disease. Of interest to the clinician is the relationship of vitamins and hormones to states of susceptibility and immunity; also the bearing of the hereditary constitution of animals upon their resistance to infection and intoxication. The discovery and use of sulphanilimide has added a great stimulus to the study of bacterial chemotherapy.

This truly great work brought strictly up to date will be found invaluable to all connected with the study of medicine in any of its various aspects and specialties.

PHYSIOTHERAPY IN MEDICAL PRACTICE: By Hugh Morris, M.D., D.M.R.E., Honorary Consulting Radiologist, Devonshire Royal Hospital, Buxton; Honorary Radiologist, Salford Royal Hospital, Royal Manchester Children's Hospital, Manchester Victoria Memorial Jewish Hospital, etc.; Radiologist, Manchester Corporation Hospital Services; Examiner in Electrotherapy to the Chartered Society of Massage; Lecturer in Electrotherapy, Schools of Massage, Salford Royal and Devonshire Royal Hospitals, etc. 276 pages with 102 illustrations. The Williams & Wilkins Company, Baltimore, Md. Price $4.50.

This book is intended for the practitioner as a guide in the personal treatment of his patients by physiotherapeutic methods or in aiding him to prescribe such treatment rather than leaving the choice and details of the method to a masseuse. Some of the principles of physics, electricity, low tension and constant currents are considered. In the chapters on the diagnosis and electrical treatment of muscle and nerve lesions diagrams of the body are given to show the chief motor points where stimulating currents should be applied.

The use of the various forms of diathermy, high-frequency currents and radiation are discussed in sufficient detail for all practical purposes. There is an interesting appendix stating precautions against shock from electro-medical apparatus, reproduced by permission of the Controller of His Majesty's Stationery Office. The book is admirably adapted to serve the purpose for which it is intended, namely, to present practical information on physiotherapy to the practitioner in a concise manner.

CLINICAL DIAGNOSIS BY LABORATORY METHODS: By James Campbell Todd, Ph.B., M.D., Late Professor of Clinical Pathology, University of Colorado, School of Medicine; and Arthur Hawley Sanford, A.M., M.D., Professor of Clinical Pathology, University of Minnesota (The Mayo Foundation); Head of Division on Clinical Laboratories, Mayo Clinic. Ninth Edition, Thoroughly Revised. 841 pages with 368 illustrations, 29 in colors. Philadelphia and London: W. B. Saunders Company, 1939. Cloth, $6.00 net.

"Todd and Sanford" as this work has been commonly spoken of for years has been a standard working manual of clinical pathology since the first edition in 1908. This ninth edition has been thoroughly revised. Old methods that have stood the test of time have been retained while new procedures have been added. Among the latter may be mentioned the technic of Bodansky for the determination of phosphate and phosphatase including his tables for calculations; also the sulphate method of Power and Wakefield is given. Among others should be mentioned the hippuric acid test for liver function; the technics for the determination of serum lipase; of cevitamic acid and sulphanilimide in both blood and urine. The Kline, Kahn, Hinton, Eagle and Kolmer tests for syphilis have been added, the technics given being those of the originators of the methods as well as the modifications. All technics are given in detail so that there should be no difficulty in carrying out any given procedure step by step. The manual is designed for students, teachers, physicians and laboratory workers.

MEDICAL OCCUPATIONS FOR GIRLS (WOMEN IN WHITE) by Lee M. Klinefelter. E. P. Dutton & Co., Inc., 300 Fourth Avenue, New York. Price $2.00.

In this volume the medical occupations for women are related

in story form. Interest is first aroused in a first aid class under the instruction of a woman physician. From there the reader is carried through all the phases of medical occupation in an absorbing conversational manner by different members of the class who contact women in these vocations. Fourteen different medical occupations are investigated and a final report is made of each. The advantages, disadvantages, preparation and training necessary in each is stressed. Data on more than 400 schools offering these courses is complete and up-to-date. Fifty-eight photographs supplement the text.

To help anyone find the work for which he or she is suited is a worthy occupation in itself. As an authority in the vocational field Mr. Klinefelter has added to his laurels in placing an authentic and informative book at the disposal of girls and women who are trying to "find" themselves in some sort of medical occupation.

NEWS AND ANNOUNCEMENTS

REGISTRY OF MEDICAL TECHNOLOGISTS
OF THE
AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

AGAIN - - A WARNING AGAINST STATE LEGISLATION

At the instigation of a commercial training school an attempt is being made to inaugurate in every state of the union a movement for legislating the vocation of medical laboratory technicians. The Registry desires to point out the danger of such a plan to the high standards that it has painstakingly set up for the status of the medical technologist if compulsory state laws were to take the place of the present non-coercive and non-political methods of ensuring the qualification of laboratory workers.

1. A certificate from the Registry of Medical Technologists of the American Society of Clinical Pathologists is valid throughout the United States and Canada recognized by Hospitals, Clinical Pathologists and Physicians. State Legislation would impose a financial burden for examination and annual renewal certificate on the applicant who already has a certificate, and a repetition of this expense in every state where he or she may desire to secure a new position.
2. Wherever legislation has been attempted the bars were always let down to graduates from commercial and non-approved schools and those who had not received a proper preliminary education as required by the Registry. No hindrance in the proposed laws was made to lay technicians conducting their own laboratories. It is frequently those very individuals who would be excluded by the Registry who are clamoring for state legislation.
3. The Registry, in adopting the voluntary system of regulating the functions of the Medical Technologist, is following the brilliant success achieved by the medical profession in maintaining the highest standards in medical schools as well as in

hospitals, not asking the aid of the State nor resorting to lobbying in state legislatures. The approval of the American Medical Association or the American College of Surgeons carries sufficient weight in the standardizing of hospitals. Similarly the certificate of the Registry which has now enrolled over half of the Medical Technologists of the country should make legislation unnecessary. In this stand the Registry has the backing of the Council on Medical Education and Hospitals of the American Medical Association which is vitally interested in the high quality of the medical laboratory personnel.

It behooves therefore every one interested in the integrity and lofty ideals of the Medical Technologists to discourage any endeavors to subject this useful and important craft to the straitjacket of political legislation.

* * *

Following quotation is from the December, 1939, issue of the "Moravian Alumnae Bulletin" of Bethlehem, Pennsylvania.

"The newly-developed course for registered technicians for which a B.S. degree or a B.A. degree with a major in science may be obtained, has graduated fourteen. In the B.S. course the hospital laboratory training of twelve months' duration may be included in the four years. This course has been fully accredited by the American Association of Clinical Pathologists. A number of hospitals have written to the College requesting information concerning the course. Eleven of these graduates have positions at the present time in pathological laboratories connected with hospitals.

"All who graduated with the degree of B.S. in Medical Technology have been placed. The pathologist of a hospital who employed one writes, 'I feel quite indebted to you for the assistance that you have rendered the hospital in securing a technician with the excellent academic background that is possessed by your candidate.' "

NATIONAL

The Board of Directors of the American Society of Medical Technologists have approved of the formation of a special representative committee to meet with the Board of Registry of the American Society of Clinical Pathologists at the time of the convention in New York next June. Matters of mutual interest will be presented at this time. Before March 1, please contact one of the members of this committee about any problems which you wish discussed at the meeting. The committee members are: Bernice Elliott, Chairman, Omaha, Nebraska; Henrietta Lyle, Lancaster, Pa.; John Conlin, Detroit, Michigan; Frieda Claussen, St. Paul, Minn.; Annette Callan, Southern Pines, North Carolina; Arthur Brice, Ross, California; Marian Baker, Lufkin, Texas; Phyllis Stanley, Newark, New Jersey; Hermine Tate, Lafayette, La.

Additional Committee Appointments for the fiscal year 1939-1940: Reception and Registration Committee—Virginia Sue Carroll, Newport News, Va.; Entertainment Committee—Marion Alcott, Boston, Mass.; Publicity Committee—Chauncey Winbigler, St. Paul, Minn.; Phyllis Stanley, Newark, New Jersey; Local Arrangements Committee—Myrtie Craver, Newark, New Jersey; Margaret Bates, Long Beach, L. I., New York; Dolores Fay, Newark, New Jersey; Marie Fortna, Hackensack, New Jersey; Sr. Reservations and Entertainment Committee—Sr. M. Theophane Carroll, Charleston, W. Va.; Sr. M. Magdalena Bero, Watertown, New York; Education and Research Committee—Henrietta Lyle, Lancaster, Pa.; Christine Seguin, Niles Center, Ill.; Exhibits Committee—Annette Callan, Chairman, Southern Pines, North Carolina; Marian Baker, Lufkin, Texas.

SCIENTIFIC EXHIBITS

Each member of the Society is invited and urged to take an active part in the exhibits. If you are planning to present an exhibit, independent of or in conjunction with a paper, please communicate at once with any of the following members of the Exhibits Committee or with the Chairman direct.

Committee—Evelyn Jardine, Mary Hitchcock Hospital, Han-

over, N. H.; Dorothy Asher Meyer, Hotel Sherry, Chicago, Ill.; Phyllis Stanley, Presbyterian Hospital, Newark, N. J.; Marian A. Baker, Taylor Clinic, Lufkin, Texas; Annette M. Callan, Chairman, Southern Pines, North Carolina.

Helpful information will be furnished later to each member planning to present an exhibit.

Plan to make this exhibit in New York City, in June, 1940, bigger and better than we have had heretofore.

DR. JAMES G. CARR AWARDED 1939 MISSISSIPPI VALLEY MEDICAL SOCIETY'S DISTINGUISHED SERVICE AWARD

Dr. James Gray Carr, A.B., M.D., F.A.C.P., of Chicago, Secretary and Professor of Medicine, Northwestern University School of Medicine, was awarded the Mississippi Valley Medical Society's Distinguished Service Award for 1939 at the recent annual meeting of the Society held at Burlington, Iowa. Dr. Carr was presented with the gold medal award and a certificate by the President of the Society, Dr. M. Pinson Neal, Prof. of Pathology, University of Missouri School of Medicine, at the annual banquet on September 28. The award is given annually to an active member of the Society for "unusual and distinguished service to the medical profession."

The citation of the Annual Award Committee of the Society in connection with Dr. Carr's Award was: "This is recognition of his innate sincerity, his deep understanding of medical problems, his kindly ministrations with a fine basic personality.

This is a most fitting tribute to a physician who has done much for the profession. A product of a small town he secured better premedical training than the average of his contemporaries which was followed by excellent medical training. This enabled him to go far as a private practitioner, consultant and teacher. Dr. Carr is still the possessor of that important quality, humility, which is the endowment of nearly all great clinicians. (Mississippi Valley Medical Journal—November, 1939).

Florida

AIM HIGH*

The objects of the Florida Society of Medical Technicians, as set forth in the Constitution, should be uppermost in all our minds. They are:

To maintain the standards of the profession.
To promote higher standards in clinical laboratory methods and research.
To elevate the status of those specializing in laboratory technique.
To promote close cooperation between the physician and the technician.

Learn to know the members of your profession and exchange ideas with them. *Each technician has something of value to contribute to the group knowledge.* Attend your local and state meetings, and lend your active assistance to earning for medical technology a recognized professional status.

* The above was taken from "The Scope", publication of the Florida Society of Medical Technicians, Vol. 1, No. 1, September, 1939. We offer our congratulations to the Florida Society on this, their initial publication, and on their ideals of organization.

Kansas

We have been informed that the Kansas State Society of Medical Technologists has been organized. Following is the program for Technician's Seminar, Hotel Jayhawk, Topeka, Kansas, December 11, 12 and 13th, 1939:

MONDAY—Call to order; Blood typing and matching, J. L. Lattimore, M.D.; Micro-blood sugar test, Allen Gold, M.T.; Recess; The Friedman test. Practical demonstration, H. C. Ebendorf, M.T.; Mechanics of Electro-cardiograms, F. C. Liscum, B.S. (Electrical Eng.); Adjourn; Blood cholesterol, Allen Gold, M.T.; Hospital routines, J. L. Lattimore, M.D.; Recess; Gastric analysis (New method), Allen Gold, M.T.; Serology, J. L. Lattimore, M.D.; Meyer's test for blood, H. C. Ebendorf, M.T.; The Ivy bleeding time technic, H. C. Ebendorf, M.T.; Adjourn.

TUESDAY—Basal metabolism, H. C. Ebendorf, M.T.; Sulfanilimide and sulfapyridine determinations, Allen Gold, M.T.; Bacteriology, N. P. Sherwood, M.D. Routine blood cultures. Stool cultures. Urine cultures. Agglutination tests. Errors in routine bacteriology; Adjourn; Spinal fluid, H. C. Ebendorf, M.T. Cell count. Differential. Sugar. Total protein. Special tests; Mechanical aids in the laboratory, J. L. Lattimore, M.D.; Recess; Calcium and phosphorus determinations, Allen Gold, M.T.; Application of tests, J. L. Lattimore, M.D.; Adjourn; BANQUET—Guest speaker, Dr. C. C. Nesselrode, Pres. Kansas Medical Society.

WEDNESDAY—The Friedman test (Rabbit autopsy, practical), H. C. Ebendorf, M.T.; Urea and NPN determinations, Allen Gold, M.T.; Recess; Sputum typing for pneumonia, J. L. Lattimore, M.D.; Bromide and Sodium chloride determination, Allen Gold, M.T.; Collection of material for toxicology, A. C. Keith, B.S.; Adjourn; Modern methods in routine tissue management, F. C. Helwig, M.D.; Hematology, C. G. Leitch, M.D. Platelet counting. Reticulocyte counting. Various anemias. Leukemia. Bone marrow examination. Special hematological methods. Including vitamin K and prothrombin times; Adjourn.

Kentucky

The Kentucky Society of Medical Technologists held its second annual meeting in Bowling Green, Ky., on September 14, 1939. Officers elected for the year beginning November 1, 1939, are as follows: President, Mary B. Leisman, Louisville; Vice-President, Robert Greene, Lexington; Secretary, Mary Elizabeth Conling, Louisville; Treasurer, David S. Silcock, Versailles; Executive Board, Nelson Blankenship, Bowling Green; O. M. Alton, Louisville. The Kentucky Society has been informed by Marian Baker, Chairman of Advisory Board of the A. S. M. T., that its application of affiliation has been approved and its charter will shortly be forthcoming. Preparations are being made to affiliate the Louisville and Lexington Societies with the State Society.

Minnesota

THE POSTGRADUATE COURSE FOR MEDICAL TECHNOLOGISTS

The first course for Medical Technologists offered by the Uni-

versity of Minnesota Center for Continuation Study was held August 31 through September 2, 1939, and was attended by 69 Medical Technologists, 46 from Minnesota, 10 from Wisconsin, 2 from North Dakota, 3 from South Dakota, 3 from Iowa, 2 from Nebraska, 1 from Kansas, 1 from Montana, and 1 from Illinois.

A souvenir in the form of a 130 page manual, "Laboratory Procedure for Medical Technologists", was given to each attending technologist. This volume may be purchased at the campus book stores for $1.15.

Pennsylvania

The seventh annual Banquet of the Pennsylvania Society of Medical Technologists was held on November 13th, 1939, in Philadelphia. The attendance surpassed all previous banquets and some Technologists from neighboring states were present.

The President, Catherine Clarkson, after her welcoming remarks, presented the toastmaster, Dr. L. W. Smith, of Temple University School of Medicine, who introduced the speakers of the evening; the Advisory Board Members: Dr. John A. Kolmer, of Temple University and Dr. Fred Boerner of the Graduate Hospital, Philadelphia; and the Honorary Members of the Society: Dr. Claude P. Brown, Dr. Stanley P. Reiman and Dr. Russell Richardson. Dr. William N. Parkinson, Dean of Temple University School of Medicine, was present and also spoke.

The guest speaker of the occasion was Dr. Armin V. St. George of the Bellevue Hospital, New York City, and the President-Elect of the American Society of Clinical Pathologists. His subject, a most timely and interesting one, was entitled: "Post Graduate Education of the Medical Technologist."

Dr. Kolmer made the announcement that the Second Technician's Institute would be held in Philadelphia the week of March 11, 1940. This news was hailed with enthusiasm and anticipation.

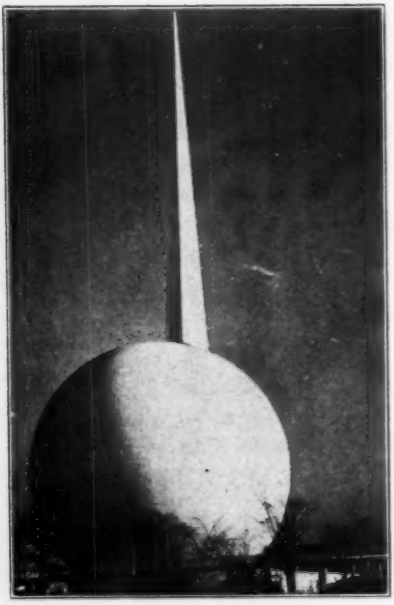
Texas

The Texas Society of Medical Technologists held its seventh annual meeting at the Hotel Adolphus, Dallas, October 6-7, 1939. Speakers on the program included:

Dr. Charles F. Carter, Dallas, Texas; Dr. Ozro T. Woods, Dallas, Texas; Mrs. Louise Hooper Cox, Denton, Texas; Dr. Buel Cairns, Dallas, Texas; Dr. J. L. Goforth, Dallas, Texas; Dr. M. D. Fulton, Dallas, Texas; Dr. William Rosenblatt, Wichita Falls, Texas; Dr. Joseph Hill, Dallas, Texas; Dr. William Potts, Dallas, Texas; Dr. Harry Wright, Austin, Texas; Miss Julia Allen, Dallas, Texas; Mr. LaMonte Tucker, Fort Worth, Texas; Dr. A. J. Gill, Dallas, Texas; Dr. George T. Caldwell, Dallas, Texas; Dr. J. H. Black, Dallas, Texas.

San Antonio was selected as the convention city for 1940.

Officers for 1940 are: President, Rose Matthaei, Houston, Texas; President-Elect, Nylah Tom, Austin, Texas; Vice-President, James Hyndehman, San Antonio, Texas; Secretary, Doris White, Waco, Texas; Treasurer, Anna Lou Smith, Forth Worth, Texas.



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ANNUAL CONVENTION

American Society of Medical Technologists

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1940

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